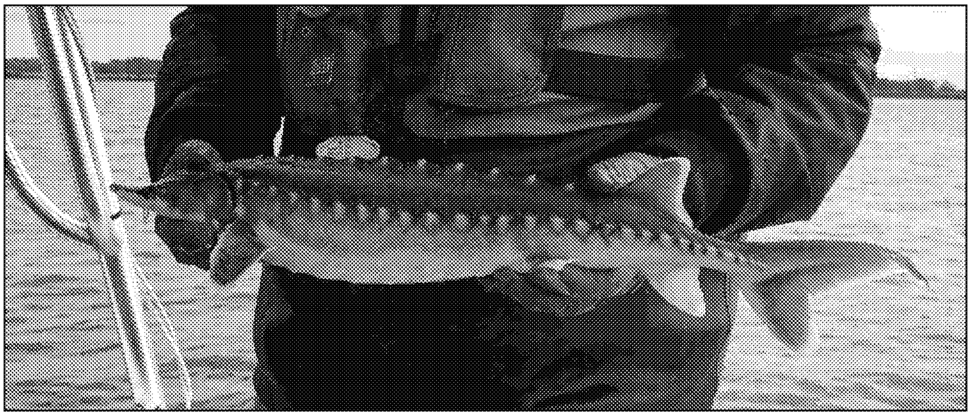
Appendix A: Atlantic Sturgeon research

DRBC supplemented the observations from the ichthyoplankton survey with NOAA Fisheries data from the Endangered Species Act Section 7 mapper for the early life stages of endangered sturgeon species. These data are summarized below and reflected in the species distribution tables within the body of this report.

Atlantic Sturgeon	**Atlantic Sturgeon**	**Shortnose Sturgeon**
Eggs and Yolk-sac Larvae	Post Yolk-sac Larvae	Post Yolk-sac Larvae
N/A	Migrating & Foraging	Migrating & Foraging
Delaware River	Delaware River	Delaware River
Acipenser oxyrinchus	Acipenser oxyrinchus	Acipenser brevirostrum
DPS: NY Bight DPS	DPS: NY Bight DPS	DPS: N/A
ESA Status: Endangered	ESA Status: Endangered	ESA Status: Endangered
Time(s) of year:	Time(s) of year:	Time(s) of year:
04/01 to 08/31	04/01 to 09/30	03/15 to 07/31
Federal Register: 77 FR 5880	Federal Register: 77 FR 5880	Federal Register: 32 FR 4001
Recovery Plan: N/A	Recovery Plan: N/A	Recovery Plan: NMFS 1998
Notes: Eggs and yolk-sac larvae may be present in spawning locations between Claymont, DE/Marcus Hook, PA and the fall line at Trenton, NJ from April 1-August 31. Timing and range are based on the spawning time/range in the Delaware River (Breece et al. 2013, p. 5-6; Simpson 2008, p. 66) plus an extra 30 days to account for the yolk-sac larvae stage.	Notes: Post yolk-sac larvae may be present in the Delaware River from the upstream limit of the spawning area at Trenton, NJ (Simpson 2008, p. 62) to the downstream limit of the saltwater line. We expect post yolk-sac larvae to be present from April 1-September 30. Timing is based on the spawning time in the Delaware River (Breece et al. 2013, p. 5; Simpson 2008, p. 66) plus an extra 60 days to account for the post yolk-sac larvae stage. DRBC has monthly spreadsheets showing where the salt line (250 mg/L) occurs. River mile (RM) 51 by DRBC RM measurement (approximately at the Hope Creek Generating Station), recorded in March 2016, is the furthest downstream location of the salt line in the past 6 years (since the beginning of 2011). However, DRBC (2013) says the average salt front range is RM 67-76 (approximately New Castle, DE to Claymont, DE).	Notes: Post yolk-sac larvae may be present in the Delaware River from the upstream limit of the spawning area in Lambertville to the downstream limit of the saltwater line (DBRC 2016). We expect post yolk-sac larvae to be present from March 15-July 31. Timing is based on the spawning time in the Delaware River (SSSRT 2010, p. 193) plus an extra 60 days to account for the post yolk-sac larvae stage.



Atlantic Sturgeon held by Ian Park, Fisheries Biologist, DNREC Division of Fish and Wildlife. This activity was conducted under a NOAA Fisheries ESA Permit No. 19255-01.

Primary literature regarding DO responses

The field of literature on Atlantic Sturgeon is somewhat limited. Approximately 15 studies have been cited on the dissolved oxygen requirements of Atlantic Sturgeon in the Delaware estuary, however only five of these studies represent novel, peer-reviewed literature articles. The remaining studies are often interpretations (or interpretations of interpretations) of these initial studies. These few novel, experimental studies on Atlantic Sturgeon are valuable contributions to understanding this species' dissolved oxygen requirements; however they do not prescribe criteria protective of Sturgeon. Due to laboratory testing limitations in the levels of dissolved oxygen treatments, age of fish, length of exposure, and coeffects of multiple parameters, it is necessary to apply professional judgment when interpreting these values. Below are the primary literature articles relating to the dissolved oxygen requirements of Atlantic Sturgeon and the closely related Shortnose Sturgeon.

Title	Author	Year
Tolerance of shortnose sturgeon, Acipenser brevirostrum, juveniles to different salinity and dissolved oxygen concentrations.	Jenkins et al.	1993
Effects of hypoxia and temperature on survival, growth and respiration of juvenile Atlantic sturgeon, Acipencer oxyrinchus.	Secor and Gunderson	1998
Bioenergetics modeling and assessment of suitable habitat for juvenile Atlantic and shortnose sturgeons in Chesapeake Bay.	Niklitschek	2001
Acute sensitivity of juvenile shortnose sturgeon to low dissolved oxygen concentrations.	Campbell and Goodman	2003
Dissolved oxygen, temperature and salinity effects on the ecophysiology and survival of juvenile Atlantic sturgeon in estuarine waters: I. Laboratory results.	Niklitschek and Secor	2009
Dissolved oxygen, temperature and salinity effects on the ecophysiology and survival of juvenile Atlantic sturgeon in estuarine waters: II. Model development and testing.	Niklitschek and Secor	2009
Experimental and field evidence of behavioral habitat selection by juvenile Atlantic Acipenser oxyrinchus oxyrinchus and shortnose Acipenser brevirostrum sturgeons.	Niklitschek and Secor	2010
An experimental approach to evaluate the effects of low dissolved oxygen acting singly and in binary combination with toxicants on larval Atlantic Sturgeon, Acipenser oxyrinchus oxyrinchus.	Wirgin and Chambers	2018

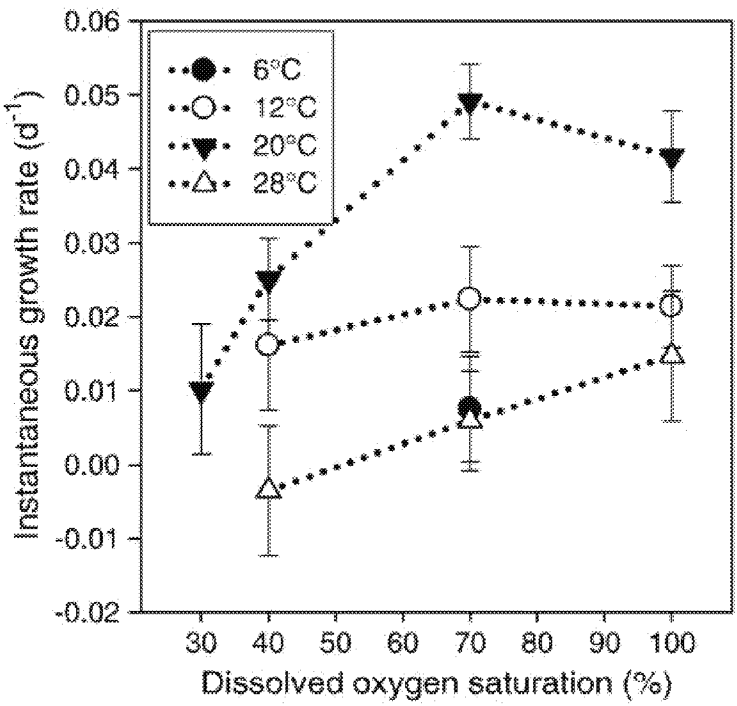
- Jenkins et al. (1993) examined the effects of short-term (6 hr) dissolved oxygen exposures to juvenile Shortnose Sturgeon (age 11-307 days) at non-stressful temperatures. The study tested a fine increment of DO levels (2.0 - 5.0 mg/L, by 0.5 mg/L), and determined values that could be considered absolute minimum tolerance levels. No mortality was seen at values greater than 4.0 mg/L and the lethal effects of levels below 4.0 mg/L were stronger in young fish than old fish.
- Secor and Gunderson (1998) performed the first DO tests on Atlantic Sturgeon. They tested the effects of hypoxia (3 vs 7 mg/L) on survival, growth, and respiration of juvenile Atlantic Sturgeon (> 10 cm total length) at stressful (26°C) and non-stressful (19°C) temperatures over 10 days. Survival, growth, and respiration were all affected by hypoxia with mortality only occurring in the 3 mg/L treatments. Timing of mortality was variable, with most mortality occurring after day one.
- Campbell and Goodman (2003) developed 24-hour LC50s for Shortnose Sturgeon. A 24-hour LC50 of 3.1 mg/L was developed for juvenile Shortnose Sturgeon at stressful temperatures. Data from this study was used by EPA (2003) to develop Criteria Minimum Concentrations for dissolved oxygen requirements of Shortnose Sturgeon (3.2 mg/L at temperatures below 29°C and 4.3 mg/L above 29°C).
- A doctoral dissertation by Niklitschek (2001) produced three important studies on the effects of hypoxia on Atlantic Sturgeon.
    - Niklitschek and Secor (2009a), a laboratory study, tested the effects of four dissolved oxygen levels (30, 40, 70, and 100% saturation) in combination with temperature and salinity over 21-day trials. At 20°C, growth rates were higher at 70% dissolved oxygen saturation vs 30% or 40% saturation. Across all temperatures, mortality was seen at all treatment levels except 100% DO saturation. Trends in mortality were dependent on temperature.
    - A follow-up paper by Niklitschek and Secor (2009b) developed a bioenergetics model incorporating the results of the previous study. According to the bioenergetics models, the effects of dissolved oxygen on growth and metabolism often plateaued at levels less than 70% saturation. When converted to concentrations these asymptotic values relate to 4.9 - 5.9 mg/L. (see Upper Threshold discussion below)
    - A third paper from the dissertation work (Niklitschek and Secor 2010), examined behavioral effects using the same experimental setup on Atlantic and Shortnose Sturgeon. Both species avoided habitats with 40% DO saturation in favor of habitats with 70 or 100% DO saturation.
- Wirgin and Chambers (2018) tested the effects of DO and toxicants on larval Atlantic Sturgeon. DO levels of 3, 4, 6, 7, 8, and 10 mg/L were tested in combinations with toxicants over 21-day trials. DO affected prey consumption, however effects on mortality were inconclusive making it difficult to draw conclusions about meaningful DO thresholds.

Upper Threshold for juvenile Atlantic Sturgeon

A closer look at the threshold above which DO no longer impacts juvenile Atlantic Sturgeon is warranted because a statement made in a research paper has been mischaracterized to suggest that a minimum DO concentration of 6.3 mg/L is required to support propagation of Atlantic Sturgeon. As listed above, two studies were published by Niklitscheck and Secor in 2009 – a laboratory study and a fish bioenergetics model that interprets and explains the laboratory results. The following statement in the first paper (Niklitschek and Secor, 2009b), which was repeated in the literature survey performed by the ANSDU (Stoklosa et al., 2018), has been grossly misrepresented.

> "For illustration purposes, if optimal growth or survival rates were used as criteria to set a hypoxia threshold for juvenile Atlantic sturgeon, that value would rise from 40 to 70% DO_{SAT} if temperature increased from 12 to 20 °C. At salinity 1 these values would correspond to concentrations of 4.3 and 6.3 mg l^{-1}, respectively."

This statement is made in the conclusions, the main point of which is that at different temperatures, juvenile Atlantic Sturgeon respond differently to the same DO saturation level. This finding is counterintuitive, since fish generally respond directly to the level of DO saturation due to the mechanism by which they extract DO from the water in their gills. The statement is illustrative and hypothetical: if 40% is your critical level of DO saturation at 12 degrees, that would be expected to change to 70% at 20 °C. The basis for the illustration is this figure (Niklitschek and Secor, 2009b, pS153):



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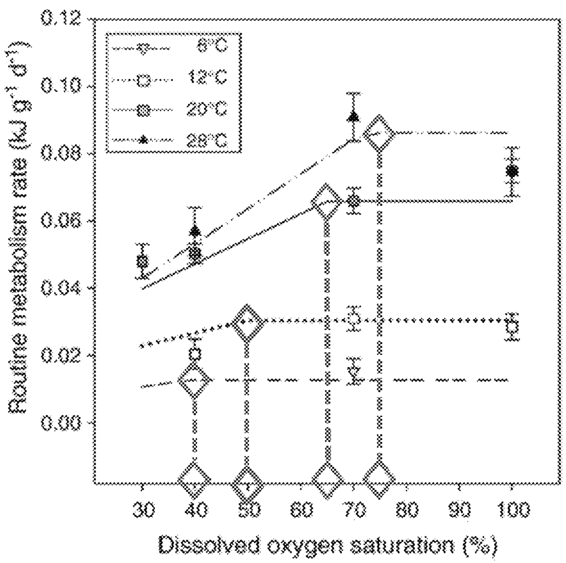
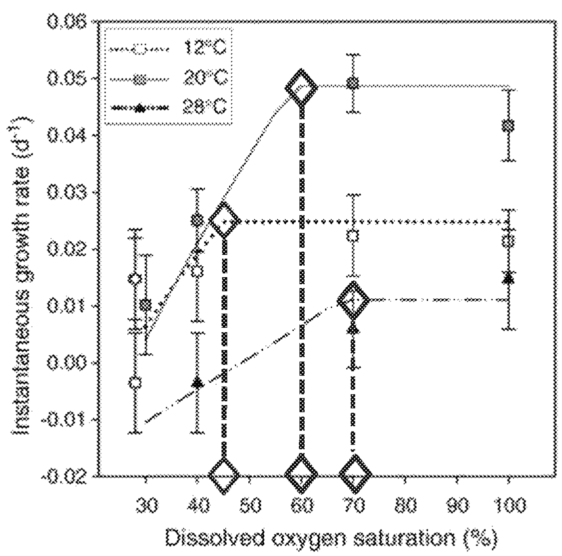
In reality of course, these are simply experimental results. They do not show the level of DO saturation that would have produced the highest growth under these particular laboratory conditions, only that 70% was better than 30% and 40%. Also, while the author references "growth and survival rates," the value of 6.3 mg/L (70% saturation at 20°C) was derived solely from the growth rate curve above, in which no further improvement in growth rate was observed from 70% to 100% saturation level (the results show a decrease in growth rate, but this is not assumed to be significant). The mortality data show conflicting results at different temperatures, but do not indicate that mortality ceases to be important once 70% saturation level is reached. The author's intent in mentioning mortality in the same sentence as growth rate appears to be to suggest that, like growth rate, the response of mortality rate to different DO saturation levels will vary depending on temperature (and salinity, for that matter).

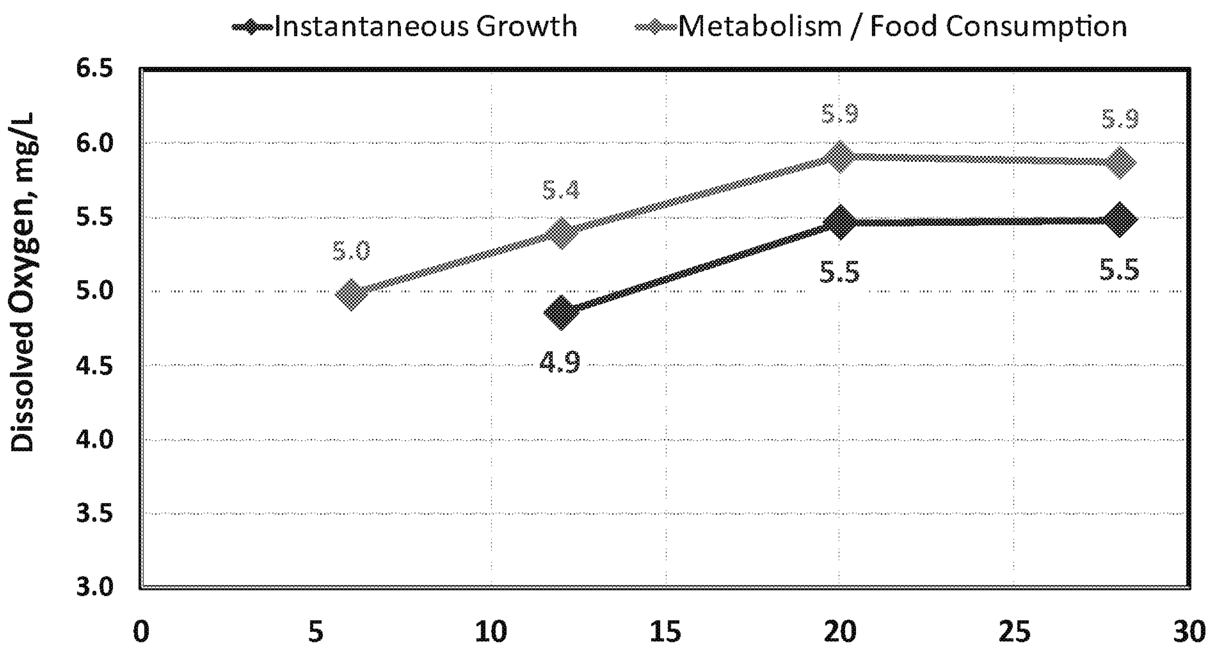
While the experimental data in the first 2009 paper are not fine enough to discern a threshold for metabolic impacts, the authors themselves developed a fish bioenergetics model to attempt to explain what might be expected between observed values. The temperature curves for routine metabolism (Niklitschek and Secor, 2009b, fig4) and growth rate (Niklitschek and Secor, 2009b, fig8), respectively, versus DO saturation level are shown below. The thresholds (identified by DRBC based on the underlying graphs) above which DO no longer impacts metabolism and growth of juvenile Atlantic Sturgeon are also shown.





These thresholds reflect the researcher's estimates, based on bioenergetics modeling and experimental findings, of the DO saturation level, at each temperature, above which metabolism and growth rate, respectively, are no longer impacted. Regarding growth rate (the basis for the 6.3 mg/L), the upper threshold level at 20°C is achieved at 60% saturation (~5.5 mg/L), not 70% (~6.3 mg/L). Interestingly, the growth rate upper threshold level at 28°C is in fact 70% saturation, which also corresponds to a DO level of 5.5 mg/L.

For a frame of reference, DRBC identified the upper threshold values for growth, metabolism, and food consumption from Niklitschek and Secor's bioenergetics study (2009b), and plotted the DO levels (assuming atmospheric pressure of 1atm and 1% salinity) against temperature in the figure below. The values in red are for growth rate and show that the upper threshold based on growth rate would be 5.5 mg/L, and that threshold concentration remains the same at 20°C and 28°C. The values for metabolism and food consumption were nearly identical and are plotted together in blue, also showing that the threshold concentration remains the same a 20°C and 28°C.



Therefore, the dissolved oxygen level above which no further benefit can be demonstrated would be 5.5 mg/L, 5.9 mg/L, or perhaps somewhere in between; it would not be 6.3 mg/L. In addition, this is an upper threshold, meaning there is no demonstrated benefit to further increases in DO. It would not be appropriate or standard practice to take an upper response threshold and set it as a minimum required value to support growth and development. Those are two different things entirely, as explained in the body of this report.